dium containing only nutrients with known chemical formulas—basically nitrate for nitrogen, sucrose for carbon, and balanced mineral salts. While they are not unique advantages, perhaps one should mention that plant tissues often grow without any change of nutrient for a month or more and that the amount of growth commonly is measured by weighing the tissue. Valuable for these tumor studies are the changes induced in certain normal cultures by interactions of auxins, kinins, and other metabolic substances. The characteristics of the callus and tumorous growths, as well as the development of roots and of stems and leaves, are influenced by varying minute concentrations. Closer examinations of single factors and of interactions are possible than could be made so far with intact plants.

It is particularly fitting that this topic should be on a program where the hosts are the New York Botanical Garden and the Rockefeller Institute. Men at both places have a long history of important fundamental biological studies. Furthermore, all but one of the speakers on this program have been or are now with one or the other of these great institutions.

A TREE TUMOR OF UNKNOWN ORIGIN

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Tumors are growths of plants, animals, or men in which the normal processes of control are, for some reason, ineffective, so that continued cell division results in massive disorganized development. When they are localized and not seriously inimical to the general functioning of the supporting body, they are called "benign." When their location or manner of development is such as to kill the supporting individual, they are called "malignant" and in man become "cancers."

Tumors may arise from many causes, including parasites and infections of many kinds. The term "cancer," however, is generally limited to malignant tumors which arise from no clearly recognizable cause and in which some one or more cells of the host body have undergone a permanent change which renders them unaffected by normal growth restraints so that the altered cell itself becomes a parasite or an infectious agent.

Tumors exist in all classes of multicellular, organized living beings. In plants, essentially malignant tumors are known to be caused by hereditary factors, by certain bacteria, and by viruses.[1] Today's discussion centers around one group of such tumors of plants, the bacterial "crown gall." My own contribution deals with a massive tumor which is not known to be a "crown gall" and, in fact, appears not to be such, yet possesses features which seem to justify careful study (Fig. 1).

"Burls" are well-known massive intumescences which occur occasionally on all sorts of trees. The best-known example is the Circassian walnut burl from which finely figured veneers for cabinet work are cut. This and many other burls are probably crown galls, having the typical irregular, fungating growth pattern resulting from shifting concentrations of bacteria in the growing layers (Fig. 2). Such burls usually occur on scattered individuals, seldom in epiphytotic numbers. Trees of the White Spruce, *Picea glauca*, however, in certain sharply limited areas, are subject to an intumescence which occurs in such numbers as to be clearly epiphytotic and which differs in structure so markedly from the usual burls that it seems doubtful whether they are crown galls.[2]

These tumors are smooth, round growths (Fig. 2). When very small, a few millimeters long on young twigs, they appear like a half-grain of rice (Fig. 4). From this they may develop into globose structures a meter or more in diameter. They occur on twigs, branches, trunks, and roots and may be single, or there may be thousands on one tree. Sections show that, irrespective of size, they *always* extend all the way to the pith (Figs. 3, 5) and hence must always arise in the bud, never from later secondary cambium.[2] On old stems a small external tumor represents the base of a narrow, acute cone of altered wood, while a large tumor represents the corresponding base of a broad, obtuse cone (Fig. 3). Each such cone is initiated by a *single cell* (Fig. 5), and the tumor trace may consist of a single file of recognizably distinct cells for 1, 2, 5, or even 20 years before it begins to expand laterally. Such files doubtless often die out and leave no external overgrowths. While these initials are always single cells, it is a common experience to find several such initials within a given region (Fig. 5), not contiguous but scattered over an area of several square millimeters of what was, at the time of initiation, procambial tissue. These intumescences thus appear to be, in fact, sectorial chimeras, arising by what seems to be a series of local somatic mutations having some common cause.

FIG. 1.—A typical rather heavily affected tree. This tree had tumors on exposed roots at distances of 6 feet and more from the base, as well as small ones on twigs and branches. It was one tree of a group of about 30 tumor-bearing individuals, about 200 feet from the shore, and in a stand mixed with white birch and Arbor Vitae, indicating a high water table.

The belief that these are sectorial chimeras of mutagenic origin is strenghtened by two physiological characteristics. First, tumor sectors are microscopically recognizable by somewhat thinner cell walls and hence slightly larger lumens; yet the cell *size* is not sufficiently different from that of adjacent normal wood to account for the massive character of the external growths. The overgrowth arises from differences not in cell size but in cell number; a growth ring of tumor wood, in an old tumor, may have ten times as many cells as the adjacent normal wood (Figs. 6, 7). This difference in number appears to result from a greatly prolonged growth season; there is evidence that the tumors are growing actively through 8–9 months of the year as compared to about 2 months for normal wood. Since growth is dependent on a suitable combination of temperature, light, soil moisture,

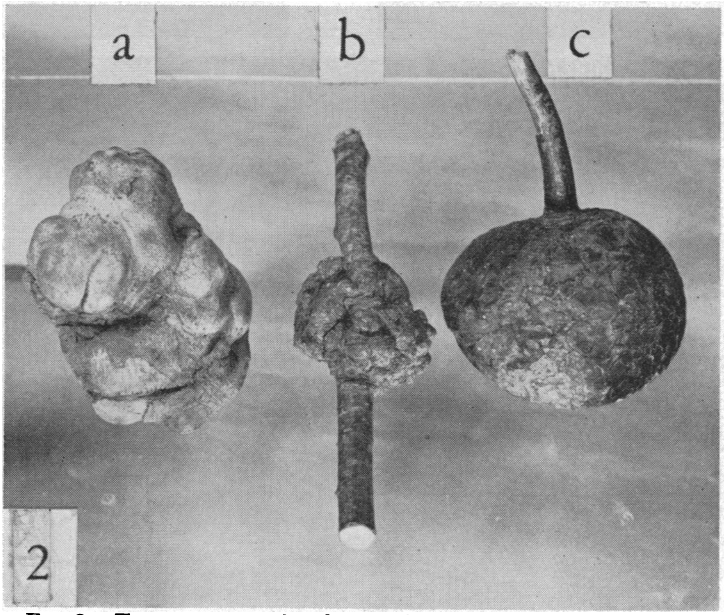

FIG. 2.—Tree tumors: *a*, beech; *b*, oak; *c*, spruce. Note that those on beech and oak have the irregular, fungating type of growth indicative of constantly shifting centers of stimulation. This is characteristic of fungus or bacterial infections and leads to the belief that these are "crown galls." The one on spruce, on the other hand, is smooth, indicating a single initial stimulus which is subsequently uniformly applied to all tumor tissue or, more probably, has become characteristic of all tumor cells.

and atmospheric humidity, of which temperature must be the most consistent variable, it seems possible that tumor cells differ physiologically from normal cells in their ability to grow well at lower temperatures than do their normal counterparts. This has not yet been tested experimentally.

Second, tissue cultures isolated from tumors are much more variable in rate of growth, growth pattern, color, and nutrient requirements than are corresponding cultures from normal wood, either adjacent to tumor wood on tumor-bearing trees or from trees which do not bear tumors. It thus appears that tumors are made up of cells which are physiologically different from normal cells both in their basic characteristics and in the instability which they show with respect to these characteristics.[3,4]

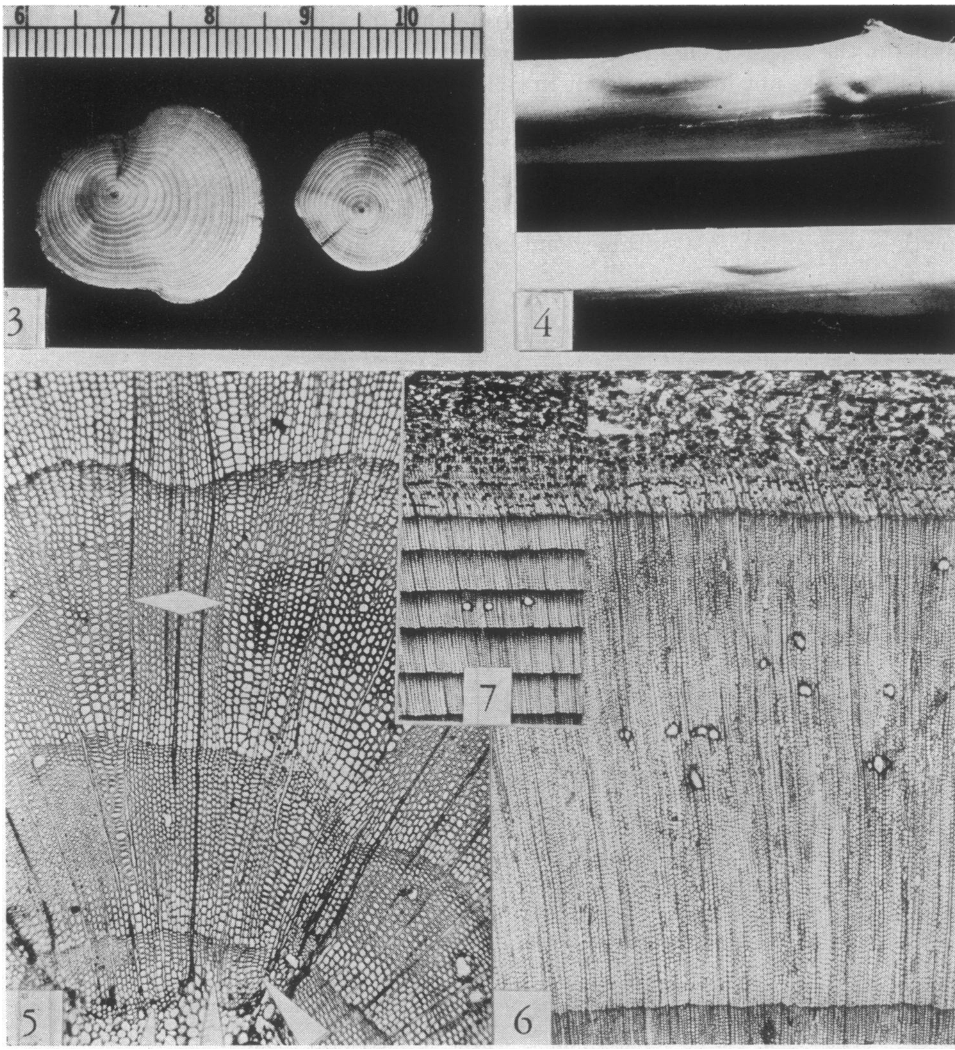

FIG. 3.—Cross-sections of two tumors from different parts of a single twig. Both can be traced to the immediate neighborhood of the pith and are hence of the same age. The difference in size is entirely attributable to different rates of *lateral* expansion, that is, to differences in the orientation of mitoses.

FIG. 4.—Examples of very small tumors. The lower one is about 6 years old. Tumors smaller than this are not externally visible under the bark of a twig but have been found sometimes (not always) by peeling twigs which show the smooth scars (absence of leaf-pegs) left after sloughing of aphid galls. Such galls live for only 1 year but may persist as dead masses for 2 or more years, leaving characteristic scars.

FIG. 5–7.—Fig. 5: A cross-section through a typical tumor. Here are three separate initials. Those at left and center developed as single files of cells for a year and a half before beginning to spread out laterally, while that at the right, adjacent to a leaf-trace, began to expand almost from the start. All three ultimately fused (beyond the area shown) to form a single intumescence. Figs. 6, 7: Sections from a tumor (Fig. 6) and from adjacent normal wood on the same stem (Fig. 7), showing the great difference in the width of the annual rings in the two regions. Note, however, that the cells in the two do not differ greatly in size, the difference resulting largely from greater cell number, due, at least in part, to the greatly prolonged growth period of tumor wood.

As previously stated, this tumor is not generally distributed in the host species but is epiphytotic in certain clearly limited regions. It occurs on trees of *Picea glauca* growing close to the seashore, especially on exposed islands and headlands along the North Atlantic seaboard from Booth Bay Harbor, Maine, to Labrador.[2] It is not found on the other two species of *Picea* native to this region, *P. rubens* and *P. nigra*. It occurs in similar regions of the North Pacific seaboard from Puget Sound to Alaska on *P. glauca* and on the closely related *P. sitchensis* but not on the inland species, *P. engelmanni*. Yet it is also reported inland on *P. glauca* in certain limited areas in the Canadian Rockies, specifically near sulfur springs, and along the north shore of Lake Superior. Affected trees are always in groups, never isolated individuals; yet such groups always include many, usually a majority, of unaffected trees. A similar tumor is reported, without information as to host species, from certain lake regions in Germany and from the French Mediterranean coast. All these areas appear to be alike in having a high water table but have no other feature so far recognized in common.

These tumors are certainly not *typical* crown galls, either in their smooth, rather than fungating, growth habit or in bearing no bacteria capable of isolation. Yet we know that crown gall is often characterized by a relatively brief sojourn of the infection, which nevertheless results in a permanent change in cell behavior, and the production of continuously growing, yet *sterile*, tumors. It is possible that the cellular change responsible for these spruce tumors might be the result of such a transient infection, restricted to the bud stage.

They are certainly not *typical* insect galls, which in no known case continue to grow after the death or departure of the insect itself. Yet the smallest tumors we have recognized have been found under the bare scars left by the sloughing-off of galls induced by the spruce-gall aphid, *Chermes abietinis*. The female of this aphid settles on the bud in the fall, inserts its suctorium into the procambial region—precisely the region in which tumors do arise—and remains in that position throughout the winter. Such an insect might thus serve either as a primary irritating agent or as a vector for an as yet undiscovered virus or as a vector for crown-gall bacteria. Or it might provide the wound needed to "trip" a genetic lesion or provide a port of entry for some chemical agent. None of these possibilities is either excluded or demonstrated by our evidence to date.

They are not *typical* irritation teratomas, as are many burls; yet their high incidence on exposed headlands with negligible numbers on nearby protected trees has strongly suggested that salt spray or salt ground water or, in the Canadian Rockies, sulfur water or other water of low oxygen content might be a major factor in their origin. This again is unproved.

Whatever their origin, they appear to offer many reasons for further study. Their epiphytotic occurrence suggests a common, hence identifiable, cause, comparable to that of the scrotal tumors of British chimney sweeps, from whose study has come our entire knowledge of chemical carcinogenesis. The woody structure of the host, with its clearly defined annual rings, permits the tracing of each tumor to its single cell of origin and enables us to date this origin so exactly that we can define rather precisely the conditions under which the tumefacient change has occurred. This we cannot do with any animal or human tumor. Their massive character permits the isolation of parallel clones of cells from adjacent normal and tumor sectors of a single individual and the physiological comparison of these cell lines in tissue cultures. While this is theoretically possible in animal and human tumors, it is fraught with such technical difficulties that it has not yet been accomplished over more than very brief periods, whereas in these spruce tumors it can be done with relative ease.

We believe that these tumors represent a promising addition to our roster of materials in which the processes of tumefaction can be profitably studied.

[1] P. R. White, *Quart. Rev. Biol.*, **26**, 1–16, 1951.
[2] P. R. White and W. F. Millington, *Cancer Research*, **14**, 128–134, 1954; *Am. J. Bot.*, **41**, 353–361, 1954.
[3] J. Reinert, *Science*, **123**, 457–458, 1956.
[4] J. Reinert and P. R. White, *Physiol. Plantarum*, **9**, 177–189, 1956.

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*A PHYSIOLOGICAL BASIS FOR AUTONOMOUS GROWTH OF THE CROWN-GALL TUMOR CELL**

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We are commemorating on this occasion the description of a rather remarkable micro-organism. This bacterium, which is now known as *Agrobacterium tumefaciens* (Smith and Town.) Conn, is responsible for the initiation of the non-self-limiting neoplastic disease of plants known as crown gall. The isolation and characterization of a tumor-inducing bacterium shortly after the turn of the century attracted considerable interest among pathologists generally because at the time of that discovery no animal tumor had yet been produced experimentally. For a period of about twenty years Erwin F. Smith made detailed comparative studies of crown-gall and malignant animal tumors and found that these two types of growth had much in common. There appeared, however, to be one fundamental difference. Smith believed that the continued unregulated proliferation of the crown-gall tumor cell was dependent upon continued stimulation by the inciting bacterium. Crown gall was, therefore, not generally accepted by oncologists as being comparable to true animal tumors because, as described by Smith, this plant disease appeared to be simply a bacterial-stimulated hyperplasia and not a truly independent growth, as are most animal cancers.

In certain plant species such as the sunflower and Paris daisy there may be produced, in addition to a primary crown-gall tumor, secondary tumors that arise at points distant from the seat of the primary growth. These secondary tumors are interesting because they are frequently free of the bacteria that initiate the primary tumor. The finding that many of the secondary tumors are bacteria-free permitted the unequivocal demonstration of the truly independent nature of the crown-gall tumor cell.[1] Sterile tissue isolated from the secondary tumors grew profusely and indefinitely on a culture medium that did not support the continued growth of normal cells of the type from which the tumor cells were derived. Small fragments of such tumor tissue implanted into a healthy host developed again into